REMARKS/ARGUMENTS

Claims 1-42 are pending in the application. Claims 31-33 have been previously withdrawn from consideration, and Claims 43-57 were previously canceled. Claims 1-32 and 34-42 stand rejected. Claims 19-20 have been deleted, Claims 1, 3, 4, 10, 11, 15, 17, 18, 21, 24, 25, 27-30, 34, 35, and 39-42 have been amended, and new Claims 58-67 have been added. Applicants now address the claim objections and rejections in the order presented in the office action.

CLAIM OBJECTIONS

Claim Objection: Claims 19 and 20 under 37 C.F.R.§1.75(c)

Claims 19 and 20 are objected to under 37 C.F.R.§1.75(c) as being improper dependent form for failing to further limit the subject matter of the previous claim. Claims 19 and 20 have been deleted, and Claims 21, 24 and 25 have been amended to delete reference to these two claims.

Claim Objection: Claims 24-26 under 37 C.F.R.§1.75(c)

Claims 24-26 are objected to under 37 C.F.R.§1.75(c) as being in improper form because a multiple dependent claim cannot depend upon any other multiple dependent claim. Claims 24 and 25 have been amended to remove improper multiple dependency, and new Claims 58-66 have been added as proper dependent claims. Claim 26 now depends from proper dependent Claim 24. Applicants respectfully request that this objection be withdrawn in view of amended Claims 24 and 25 and new Claims 58-66.

CLAIM REJECTION: CLAIMS 1-30 AND 34-42 UNDER 35 U.S.C. §112, 2ND PARAGRAPH

Claims 1-30 and 34-42 stand rejected under 35 U.S.C. §112, 2nd Paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

Claim Rejection: Claims 1, 15, 29, 27 and 39

The phrase "at the time of consumption" found in Claims 1, 15, 29, 27 and 39 has been considered indefinite because it is unclear what the metes and bounds of the limitation are.

Applicants appreciate the Examiner's comments and agree that transgene under the control of a constitutive promoter would promote expression of the transgene constitutively.

Applicants have amended Claims 1, 15, 27, 29 and 39 to delete this phrase, and respectfully request that this rejection be withdrawn.

Claim Rejection: Claims 3, 4, 10, 11, 17, 18, 28, 30, 35, 40 and 42

Claims 3, 4, 10, 11, 17, 18, 28, 30, 35, 40 and 42 have been rejected as indefinite because the phrase "said resveratrol synthase transgene is SEQ ID NO:2" in Claims 3, 4, 10, 11, 17 and 18 and the phrase "said open reading frame is SEQ ID NO:2" in Claims 28, 30, 35, 40 and 42 refer to a transgene but SEQ ID NO:2 is an amino acid sequence encoded by the transgene.

As suggested by the Examiner, Applicants have amended Claims 3, 4, 10, 11, 17, 18, 28, 30, 35, 40 and 42 to indicate the transgene or open reading frame encodes SEQ ID NO:2. Applicants request that this rejection be withdrawn in view of amended Claims 3, 4, 10, 11, 17, 18, 28, 30, 35, 40 and 42.

Claim Rejection: Claims 27, 34 and 41

In Claims 27, 34 and 41, the phrase "under conditions conducive to the accumulation of ..." has been considered indefinite because it is unclear what the metes and bounds of the limitation are.

Applicants traverse this rejection. Claims 27, 34 and 41 specify the plant cells are transformed with a resveratrol synthase transgene under control of a constitutive promoter and that the transgenic plant cells are cultivated under conditions conducive to the accumulation of p-coumaryl CoA and malonyl CoA. Both p-coumaryl CoA and malonyl CoA are known precursors of many metabolites in plants, including phenylpropanoids, flavonoids, lignin and fatty acids, and conditions conducive to the accumulation of these precursors are well known in the art for all precursors to include keeping the plants in a healthy non-stressed state. At Page 18, lines 10-15, Applicants disclose the accumulation of resveratrol glucoside is favored in non-stressed tissues. Also, acetyl-CoA (ACC) carboxylase catalyzes the ATP-dependent carboxylation of acetyl CoA to produce malonyl CoA in plants, and increased activity of ACC is well known in the art to be associated with optimal lighting (abstract provided at Tab A: Hunter, SC and Ohlrogge , JB. 1998. "Regulation of spinach chloroplast acetyl-CoA carboxylase," *Arch Biochem Biophys* 359:170-178). For synthesis of p-coumaryl CoA from phenylalanine (which all plants have), three enzyme steps are required: PAL (phenylalanine ammonia lyase; CA4H (cinnamate-4-hydroxylase); and CL (coumaryl-CoA ligase). The activity of these enzymes are well known in the art to increase when plants are grown in the light (at Tab B: Hahlbrock, K and

Grisebach, H. 1970. "Formation of coenzyme a esters of cinnamic acids with an enzyme preparation from cell suspension cultures of parsley," *FEBS Lett* 11:62-64, abstract only; Bell-Lelong, et al. 1997. "Cinnamate-4-hydroxylase expression in Arabidopsis. Regulation in response to development and the environment," *Plant Physiol* 113:729-738; and Kubasek, et al. 1992. "Regulation of flavonoid biosynthetic genes in germinating Arabidopsis seedlings," *Plant Cell* 4:1229-1236). Thus, Applicants believe that this limitation is defined in their specification and by common knowledge for one skilled in the art, and request that this rejection be withdrawn.

Claim Rejection: Claim 29

Claim 29 has been considered indefinite for failure to set forth positive method steps by which one skilled in the art is to practice the claimed method.

Claim 29 has been amended to include positive steps of "transforming" plant cells of an edible and "consuming" the edible plant." Applicants request that this rejection be withdrawn in view of amended Claim 29.

Claim Rejection: Claim 34

In Claim 34, the limitation "cultivating...under conditions to...the suppression of β-glucosidases" has been considered indefinite because it is unclear what the metes and bounds of such conditions are.

Claims 27, 34 and 41 have been amended, and Applicants traverse this rejection as it may be applied to the amended claims. Claims 27, 34 and 41 specify the plant cells are transformed with a resveratrol synthase transgene under control of a constitutive promoter and that the transgenic plant cells are cultivated under conditions conducive to...minimizing the concentration of β-glucosidases active on resveratrol glucoside. It is well known in the art that β-glucosidases are stored in plants cells or compartments separated from the cells or compartments which contain the glucosides of natural products; and when the cells are crushed or stressed (such as by fungal infection, UV damage, or wounding), the compartments are no longer separated, resulting in the release of the β-glucosidases and their subsequent action on the natural glucoside products in other cells or compartments (references provided at Tab C: light decreases the accumulation of β-glucosidases and damaging tissue allows the β-glucosidases to act on their substrates in Cicek, M. and Esen, A. 1998. "Structure and expression of a Dhurrinase (β-glucosidase) from

sorghum," *Plant Physiol* 116:1469-1478; damaging tissues initiates hydrolysis in White, et al. 1998. "Cyanogenesis in cassava," *Plant Physiol* 116:1219-1225; and compartmentation and β-glucosidase activity detailed in Poulton, JE and Li, CP. 1994. "Tissue level compartmentation of (*R*)-amygdalin and amygdalin hydrolase prevents large-scale cyanogenesis in undamaged *Pruna* seeds," *Plant Physiol* 104:29-35). Further, Applicants disclose that the accumulation of resveratrol glucoside is favored in plant tissues with low levels of β-glucosidases and that low levels of β-glucosidases are more likely to be present in non-stressed tissues (Page 18, lines 10-15). Thus, Applicants believe that this limitation is defined in their specification and by common knowledge for one skilled in the art, and request that this rejection be withdrawn.

CLAIM REJECTION: CLAIMS 3-7, 10-14, 17, 18, 21-26, 28, 30, 35-38, 40 AND 42 UNDER 35 U.S.C. §112, 1ST PARAGRAPH

Claims 3-7, 10-14, 17, 18, 21-26, 28, 30, 35-38, 40 and 42 stand rejected under 35 U.S.C. §112, 1st paragraph, with respect to the claims pertaining to transformed plant material, compositions comprising said transformed plant material, transformed plants, and a method of using said transformed plants comprising a transgene encoding the amino acid sequence of SEQ ID NO:2. In particular, the Examiner states that while the specification discloses use of the peanut resveratrol synthase (RS) cDNA as taught by Tropf et al. (*J Mol Evol* 38:610-618, 1994), which encodes a protein beginning with the amino acids MVSVSGIRK (or MetValSerValSerGlyIleArgLys), Applicants teach the peanut RS3 gene encodes a protein beginning with the amino acids MEGGIRK (or MetGluGlyGlyIleArgLys). For this reason, the Examiner contends that it is unclear from the present specification that the instant claims are enabled, and further, it is unclear why there is a difference between the teachings of the amino acid sequence of the peanut RS3 protein as taught by Tropf and the present invention.

Applicants traverse this rejection as it may be applied to Claims 5-7, 12-14, 22-23, 26 and 36-38 and amended Claims 3, 4, 10, 11, 17, 18, 21, 24, 25, 28, 30, 35, 40 and 42.

According to Tropf et al (Page 611, Col 2, 3rd paragraph), the original constructs for expression of stilbene synthase from peanut (RS3) was described by Lanz et al (at Tab D: Lanz et al. 1991. *J Biol Chem* 266:9971-9976) which outlines the construction of pRS3. As specified by Lanz et al. (Page 9972, Col 2, line 6-11), RS3 (described in Lanz et al. 1990. *Planta* 181:160-175 at Tab E) is a 2.7 kilobase pair genomic *Eco*RI fragment from peanut that, except for a few

missing base pairs at the amino-terminal end (including the start codon ATG for Met or M), contains the complete protein coding region, which is split by a single intron. This initial RS3 N-terminal sequence lacking the start codon is shown in Fig. 2 of Lanz, 1990, and begins with the sequence **GAATTC**GC. This sequence was adapted for expression of active RS protein by several steps. These included removing the intron sequence, producing the same sequence in that part of the coding region as would be obtained for a cDNA clone corresponding to this gene. The modified coding region was then transferred into the EcoRI/BamHI site of pINIIA3 (Nakamura and Inouye. 1982. *EMBO J* 1:771-75), resulting in the fusion of 3 amino acids from the *E. coli* prolipoprotein sequence in frame to the amino terminus of the RS3 coding region. This was then moved to a derivative of pTZ19R, which allowed inducible expression of the modified but active STS (RS) encoded by pRS3 in *E. coli*, , resulting in the sequence of ... TATTAATAATGAAA-GGGG**GAATTC**GC... . The resulting DNA and amino acid sequences are shown in Lanz et al (1991), Figure 3.B. At this stage, the first 6 amino acids of the recombinant STS (RS) protein are MKGGIR (or MetLysGlyGlyIleArg).

On Page 611, last sentence, Tropf et al. states that the STS from *A. hypogaea* was recloned into pQE-6 after introduction of a *Nco*I site into the start ATG by site-directed mutagenesis. An *Nco*I site contains the sequence 5'-CCATGG-3'; all 6 bases must be present in this order. If the site includes the start ATG codon (M or Met) of a protein, and the initial sequence does not already contain a G after the ATG, the change of this base from C, A, or T to G will result in the change of the amino acid following the initial M (or Met) residue. In this case, introduction of a *Nco*I site changes the sequence from ATAATGAAA to ACCATGGAA (...TATTAACCATGGAAGGGG**GAATTC**GC...), changing the second amino acid from K or Lys (AAA codon) to Glu or E (GAA codon). According to Tropf, the resulting recombinant protein beginning with MEGGIRK (or MetGluGlyGlyIleArgLys) was an active STS (RS) enzyme.

While Tropf discloses several different RS sequences, one of the recombinant proteins referenced by Tropf, the preparation of which is described above, is the same sequence disclosed in the present application as SEQ ID NO:2. Moreover, Applicants indicated in the specification that restriction enzyme digest and the DNA sequencing was used to confirm the integrity of the construct (Specification, Page 20, Line 26-27), and although not presented in the specification,

Applicants confirmed that the clone encoded an active resveratrol synthase enzyme by expression in *E. coli* followed by enzyme assays in their laboratory.

For reasons given above, Applicants believe that Claims 3-7, 10-14, 17, 18, 21-26, 28, 30, 35-38, 40 and 42 are enabled, and request that this rejection be withdrawn.

CLAIM REJECTION UNDER 35 U.S.C. §103

Claims 1, 2, 5-9, 12-16, 19-27, 29, 34, 36-39 and 41 under 35 U.S.C. §103(a) as being unpatentable over Schroder et al (USPN 5,689,046; hereinafter referred to as "Schroder") in view of Comai et al (USPN 5,106,739; hereinafter referred to as "Comai")

The Examiner states that Schroder teaches a method of producing a transgenic plant comprising a resveratrol synthase transgene and transformed plants, in particular, a method of producing a transgenic alfalfa plant comprising the resveratrol synthase transgene and desirability of transforming soybean with the resveratrol synthase transgene. While the Examiner states that Schroder teaches that other promoter sequences besides promoter sequence native to the taught resveratrol synthase gene can be operably linked to the taught coding sequence, it is recognized that Schroder does not specifically teach operably linking the taught resveratrol synthase coding sequence to a constitutive promoter. The Examiner contends that Comai teaches a CAMV 35S enhanced MAS promoter that is constitutive and functional in transgenic plants, and that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of Applicants' invention to use the teachings of Comai to use the constitutive enhanced MAS promoter to modify the teachings of Schroder to constitutively express a resveratrol synthase transgene. Finally, the Examiner contends that Applicants' "edible plant material" is considered an intended use limitation and that Applicants' composition comprising the edible plant material would have been obvious given that Schroder motivates one of ordinary skill in the art to transform plants commonly used as human and animal food products.

Applicants traverse this rejection to the extent that it is applied to Claims 2, 5-9, 12-14, 16, 22, 23, 26, and 36-38; amended Claims 1, 15, 21, 24, 25, 27, 29, 34, 39 and 41; and new Claims 58-66. Claims 19-20 have been deleted.

Comai teaches high levels of expression of coding sequences obtained by using CAMV 35S enhanced MAS promoter in plant cells but does not teach or suggest its use in transformation of plants with a stilbene synthase gene.

Schroder teaches transformation of plants with a stilbene synthase gene for the purpose of increasing the resistance of the plant to a pest. At Col 2, lines 46-56, Schroder defines "stilbene synthase" as every enzyme which brings about the synthesis of those plant substances defending against pests (phytoalexines) and containing the stilbene skeleton; and lists preferred stilbenes: 3,5-dihydroxystilbene; 3,5-dimethoxy-4'-hydroxystilbene; 3,3',5-trihydroxystilbene (resveratrol). The example given in the specification shows transformations using a stilbene synthase gene isolated from peanut under the control of an inducible promoter to accumulate stilbene synthase product upon induction. At Col 1, lines 25-29, Schroder states that stilbenes are present in only very low concentrations in healthy tissue, but in very high amounts at the point of infection following infection or wounding. Moreover, Schroder teaches that the stilbene synthase gene is expressed upon exposure to a pest (Claim 1, 8, and 15), i.e., upon exposure to stress. At Col 18, lines 20-24, Schroder states that the presence of stilbene synthase and stilbenes were detected in transformed plants with the aid of specified antibodies; however, data is not presented to indicate what stilbene(s) were actually detected. However, Schroder et al reported in an earlier paper that two cDNA clones encoding the same resveratrol synthase as disclosed in his later patent catalyzes the formation of resveratrol upon induction (at Tab F: Schroder et al. 1988. *Eur J Biochem* 172:161-169), leading one skilled in the art to expect the accumulation of resveratrol from the resveratrol synthase gene.

When the teachings of Schroder are combined with a constitutive promoter as taught by Comai, the predicted result based on his patent and earlier publication would be free resveratrol constitutively produced in transgenic plants. There is nothing in either Schroder or Comai singly or combined that suggests the accumulation of resveratrol glucoside in a somewhat tissue-specific manner as demonstrated in the present invention. Moreover, there is nothing in Schroder or Comai, singly or combined, that would lead someone skilled in the art to contemplate that when a plant which does not contain an endogenous resveratrol synthase gene is transformed with a resveratrol synthase gene or cDNA, an endogenous glucosyl transferase would be able to interact with the product of the resveratrol synthase transgene to form resveratrol glucoside. Schroder or Comai, singly or combined, also do not teach the increased nutritional or nutraceutical value obtained by the unexpected accumulation of resveratrol glucoside in plants expressing a resveratrol synthase gene under the control of a constitutive promoter.

In the present invention, Applicants have shown that transforming plant cells with a resveratrol synthase transgene under the control of a constitutive promoter results in the accumulation of not resveratrol, but instead resveratrol glucoside upon expression of the resveratrol synthase transgene (Figs. 3A, 4-6 and 8 for alfalfa and Figs. 15 and18 in transformed soybean). Moreover, Applicants have shown that the resveratrol glucoside formation is tissue-specific with high accumulation in young leaves and old internodes of transformed alfalfa (Specification, Page 23, lines 17-19). Applicants also disclose that accumulation of resveratrol glucoside is favored in plant tissues which contain the necessary biosynthetic precursors and low levels of β-glucosidases; and that such conditions may be found in any plant cell, but are more likely to be present in non-stressed tissues, such as leaves constantly expressing a resveratrol synthase coding region driven by a constitutive promoter, rather than wounded or infected tissues which contain, release, or induce β-glucosidases (Page 18, lines 10-15).

In summary, Schroder, either alone or combined with Comai, does not teach or suggest that transforming a plant with resveratrol synthase results in the accumulation of resveratrol glucoside as taught by the present invention. For these reasons, Applicants respectfully request that this rejection be withdrawn.

Claims 1, 2, 5-9, 12-16, 19-27, 29, 34, 36-39 and 41 under 35 U.S.C. §103(a) as being unpatentable over Hain et al (USPN 5,689,047; hereinafter referred to as "Hain") in view of Comai et al (USPN 5,106,739; hereinafter referred to as "Comai")

The Examiner states that Hain teaches a method of producing a transgenic plant comprising a resveratrol synthase transgene and transformed plants, in particular, the desirability of transforming alfalfa and soybean comprising the taught resveratrol synthase transgene. While the Examiner states that Hain teaches that other promoter sequences besides promoter sequence native to the taught resveratrol synthase gene can be operably linked to the taught coding sequence, it is recognized that Hain does not specifically teach operably linking the taught resveratrol synthase coding sequence to a constitutive promoter. The Examiner contends that Comai teaches a CAMV 35S enhanced MAS promoter that is constitutive and functional in transgenic plants, and that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of Applicants' invention to use the teachings of Comai to use the constitutive enhanced MAS promoter to modify the teachings of Hain to constitutively express a resveratrol synthase transgene. Finally, the Examiner contends that Applicants' "edible plant material" is

considered an intended use limitation and that Applicants' composition comprising the edible plant material would have been obvious given that Hain motivates one of ordinary skill in the art to transform plants commonly used as human and animal food products.

Applicants traverse this rejection to the extent that it is applied to Claims 2, 5-9, 12-14, 16, 22, 23, 26, and 36-38; amended Claims 1, 15, 21, 24, 25, 27, 29, 34, 39 and 41; and new Claims 58-66. Claims 19-20 have been deleted.

Comai teaches high levels of expression of coding sequences obtained by using CAMV 35S enhanced MAS promoter in plant cells but does not teach or suggest its use in transformation of plants with a stilbene synthase gene.

Hain teaches transformation of plants with a stilbene synthase gene derived from grapevines for the purpose of increasing the resistance of the plant to a pest. At Col 2, lines 41-43, Hain discloses resveratrol as a preferred stilbene with resveratrol synthase as a preferred stilbene synthase. The examples given in the specification shows transformations using stilbene synthase genes isolated from grapevine under the control of an inducible promoter to accumulate stilbene synthase product upon induction. At Col 2, lines 34-42, Hain defines stilbene synthase as any enzyme which causes the formation of vegetable defense substances against pests (phytoalexins) which show a stilbene skeleton; and resveratrol is listed as the preferred stilbene in the application. At Col 14, lines 2-30, Hain states that the presence of stilbene synthase and stilbenes were detected in transformed plants with the aid of specified antibodies; however, data is not presented to indicate what stilbenes were actually detected. However, Hain et al reported in an earlier paper that transforming tobacco plants with the same grapevine stilbene synthase genes as disclosed in his later patent catalyzes the formation of resveratrol (at Tab G: Hain at el. 1993. *Nature* 361:153-156), leading one skilled in the art to expect accumulation of resveratrol.

When the teachings of Hain are combined with a constitutive promoter as taught by Comai, the predicted result based on his patent and earlier publication would be free resveratrol constitutively produced in transgenic plants. There is nothing in either Hain or Comai singly or combined that suggests the accumulation of resveratrol glucoside in a somewhat tissue-specific manner as demonstrated in the present invention. Moreover, there is nothing in Hain or Comai singly or combined that would lead someone skilled in the art to contemplate that when a plant which does not contain an endogenous resveratrol synthase gene is transformed with a resveratrol synthase gene or cDNA, an endogenous glucosyl transferase would be able to interact

with the product of the resveratrol synthase transgene to form resveratrol glucoside. Hain or Comai, singly or combined, also do not teach the increased nutritional or nutraceutical value obtained by the unexpected accumulation of resveratrol glucoside in plants expressing a resveratrol synthase gene under the control of a constitutive promoter.

In the present invention, Applicants have shown that transforming plant cells with a resveratrol synthase transgene under the control of a constitutive promoter results in the accumulation of not resveratrol, but instead resveratrol glucoside upon expression of the resveratrol synthase transgene (Figs. 3A, 4-6 and 8 for transformed alfalfa and Figs. 15 and18 in transformed soybean). Moreover, Applicants have shown that the resveratrol glucoside formation is tissue-specific with high accumulation in young leaves and old internodes of transformed alfalfa (Specification, Page 23, lines 17-19). Applicants also disclose that accumulation of resveratrol glucoside is favored in plant tissues which contain the necessary biosynthetic precursors and low levels of β-glucosidases; and that such conditions may be found in any plant cell, but are more likely to be present in non-stressed tissues, such as leaves constantly expressing a resveratrol synthase coding region driven by a constitutive promoter, rather than wounded or infected tissues which contain, release, or induce β-glucosidases (Page 18, lines 10-15).

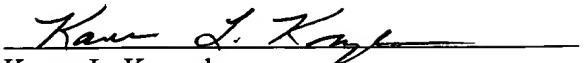
In summary, Hain , either alone or combined with Comai, does not teach or suggest that transforming a plant with resveratrol synthase results in the accumulation of resveratrol glucoside as taught by the present invention. For these reasons, Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

Applicants do not believe that any additional claim fee is due at this time. If this is in error, Applicants request that the necessary fee be deducted from the Sidley Austin Brown & Wood Deposit Account No. 18-1260.

Any fee required by this document other than the issue fee, and not submitted herewith should be charged to Sidley Austin Brown & Wood LLP's Deposit Account No. 18-1260. Any refund should be credited to the same account.

Respectfully submitted,



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November 24, 2003

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